

Draft Genome Sequence of the Methicillin-Resistant *Staphylococcus* aureus Isolate MRSA-M2

Janette M. Harro,^a Sean Daugherty,^b Vincent M. Bruno,^{b,c} Mary Ann Jabra-Rizk,^d David A. Rasko,^{b,c} Mark E. Shirtliff^{a,c}

Department of Microbial Pathogenesis, School of Dentistry, University of Maryland, Baltimore, Maryland, USAa; Institute of Genome Sciences, University of Maryland, School of Medicine, Baltimore, Maryland, USAb; Department of Microbiology and Immunology, School of Medicine, University of Maryland, Baltimore, Maryland, USAc; Department of Oncology, Diagnostic Sciences, School of Dentistry, University of Maryland, Baltimore, Maryland, USAd

We report the draft genome sequence of a methicillin-resistant strain of *Staphylococcus aureus*, designated MRSA-M2. This clinical isolate was obtained from an osteomyelitis patient undergoing treatment at the University of Texas Medical Branch (Galveston, TX). This strain is an ST30, *spa* type T019, *agr* III strain and has been utilized as a model *S. aureus* strain in a number of proteomic, transcriptomic, and animal model studies.

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Staphylococcus aureus is among the leading pathogens causing bloodstream infections able to form biofilms on host tissue and indwelling medical devices and to persist and cause disease (1). Infections caused by *S. aureus* are becoming more difficult to treat due to an increasing resistance to antibiotics. In addition to genetic mechanisms of antibiotic resistance, *S. aureus* isolates often have the ability to form biofilms, making them even more resistant to treatment (2). *S. aureus* M2 has been used as a model *S. aureus* strain in a number of proteomic, transcriptomic, and animal model studies (3–9) and has shown interkingdom interactions between *S. aureus* and *Candida albicans* (10, 11) and the potential importance of quorum sensing in pathogenesis of both organisms in animal model hosts (10, 11).

The S. aureus M2 strain was obtained from an osteomyelitis patient undergoing treatment at the University of Texas Medical Branch (Galveston, TX) (3, 9). This strain is an ST30, spa type T019, agr III strain. Following isolation, the strain was stored at -70° C in defibrinated sheep blood and grown on tryptic soy broth (TSB) and tryptic soy agar prior to genomic DNA extraction. Genomic DNA was isolated from an overnight culture using the Qiagen genomictip Sigma GenElute kit (Qiagen) with the addition of lysostaphin (100 μg/ml) and incubation at 37°C for 1 h in the initial step (Sigma-Aldrich). The genomic DNA was sequenced at the University of Maryland School of Medicine, Institute for Genome Sciences, Genome Resource Center (http://www.igs.umaryland.edu/). The genome sequence of S. aureus M2 was generated using paired-end libraries with 300-bp inserts on the Illumina HiSeq2000. The draft genome data were assembled using the Velvet assembly program (12). The resulting genome assembly contained 133 contigs that were longer than 500 bp. This results in a predicted genome size of 2,902,463 bp with an average G+C% of 32.12 (range 25.69 to 51.17%). Both of these parameters are similar to those reported for previous S. aureus genome projects (http://gscid.igs.umaryland.edu/wp.php?wp=03genome _analysis_of_the_staphylococcus_aureus_complicated_infection **_group**). The contig data were annotated using the Annotation

pipeline at the Institute for Genome Sciences, Informatics Resource Center (http://www.igs.umaryland.edu/). The predicted genes from the draft genomes were also similar to previously sequenced *S. aureus* genomes, with 2,725 genes. This predicted gene count is similar to those of other *S. aureus* genomes in the public domain.

The genomic features associated with the previously observed phenotypes of the M2 isolate, such as sequence type ST30, spa type T019, and an agr III phenotype, were each identified and verified in the draft genome sequence. The icaADBC operon was also confirmed in the sequence. The icaADBC locus mediates poly-N-acetyl- β -1,6-glucoamine assembly into the polysaccharide intercellular adhesin (13) that regulates biofilm formation in multiple S. aureus strains (14). Isolates of the agr III phenotype, which lack a functional agr system, are identified as medium biofilm producers due to icaAR and rsbU expression during late- and postexponential growth phases (15).

Further molecular-based studies are under way with the *S. aureus* M2 isolate that will advance our understanding of *S. aureus* pathogenesis.

Nucleotide sequence accession number. The genome data have been deposited in GenBank with accession number AMTC00000000.

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REFERENCES

1. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, Craig AS, Zell ER, Fosheim GE, McDougal LK, Carey RB, Fridkin SK. 2007. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 298:1763–1771.

- Stewart PS, Costerton JW. 2001. Antibiotic resistance of bacteria in biofilms. Lancet 358(9276):135–138.
- Shirtliff ME, Calhoun JH, Mader JT. 2002. Experimental osteomyelitis treatment with antibiotic-impregnated hydroxyapatite. Clin. Orthop. Relat. Res. 401:239–247.
- Leid JG, Shirtliff ME, Costerton JW, Stoodley P. 2002. Human leukocytes adhere to, penetrate, and respond to Staphylococcus aureus biofilms. Infect. Immun. 70:6339–6345.
- Brady RA, Leid JG, Camper AK, Costerton JW, Shirtliff ME. 2006. Identification of *Staphylococcus aureus* proteins recognized by the antibody-mediated immune response to a biofilm infection. Infect. Immun. 74:3415–3426.
- Brady RA, Leid JG, Kofonow J, Costerton JW, Shirtliff ME. 2007. Immunoglobulins to surface-associated biofilm immunogens provide a novel means of visualization of methicillin-resistant *Staphylococcus aureus* biofilms. Appl. Environ. Microbiol. 73:6612–6619.
- Brady RA, O'May GA, Leid JG, Prior ML, Costerton JW, Shirtliff ME. 2011. Resolution of *Staphylococcus aureus* biofilm infection using vaccination and antibiotic treatment. Infect. Immun. 79:1797–1803.
- 8. Prabhakara R, Harro JM, Leid JG, Harris M, Shirtliff ME. 2011. Murine immune response to a chronic *Staphylococcus aureus* biofilm infection. Infect. Immun. **79**:1789–1796.
- 9. Prabhakara R, Harro JM, Leid JG, Keegan AD, Prior ML, Shirtliff ME.

- 2011. Suppression of the inflammatory immune response prevents the development of chronic biofilm infection due to methicillin-resistant *Staphylococcus aureus*. Infect. Immun. **79**:5010–5018.
- Peters BM, Ovchinnikova ES, Krom BP, Schlecht LM, Zhou H, Hoyer LL, Busscher HJ, van der Mei HC, Jabra-Rizk MA, Shirtliff ME. 2012. Staphylococcus aureus adherence to *Candida albicans* hyphae is mediated by the hyphal adhesin Als3p. Microbiology 158:2975–2986.
- 11. Peters BM, Jabra-Rizk MA, Scheper MA, Leid JG, Costerton JW, Shirtliff ME. 2010. Microbial interactions and differential protein expression in *Staphylococcus aureus-Candida albicans* dual-species biofilms. FEMS Immunol. Med. Microbiol. **59**:493–503.
- 12. **Zerbino DR, Birney** E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. 18:821–829.
- 13. Cramton SE, Gerke C, Schnell NF, Nichols WW, Götz F. 1999. The intracellular adhesion (*ica*) locus is present in *Staphylococcus aureus* and is required for biofilm formation. Infect. Immun. 67:5427–5433.
- Cafiso V, Bertuccio T, Santagati M, Demelio V, Spina D, Nicoletti G, Stefani S. 2007. agr-genotyping and transcriptional analysis of biofilmproducing *Staphylococcus aureus*. FEMS Immunol. Med. Microbiol. 51: 220–227.
- Beenken KE, Dunman PM, McAleese F, Macapagal D, Murphy E, Projan SJ, Blevins JS, Smeltzer MS. 2004. Global gene expression in Staphylococcus aureus biofilms. J. Bacteriol. 186:4665–4684.